PROTEIN CATALYZED CAPTURE AGENT BASED BIOSENSOR FOR THE DETECTION OF SARS-COV-2

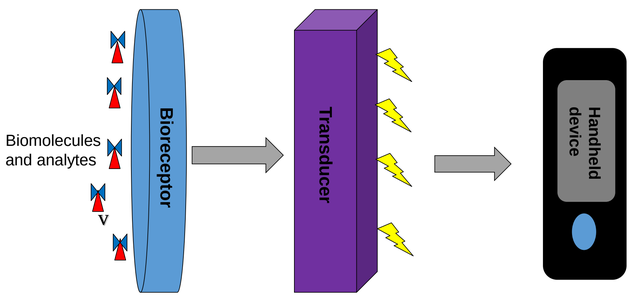
ALEXIS FORD† AND DR. SANCHAO LIU‡,
† DEPARTMENT OF CHEMISTRY AND PHYSICS, UNIVERSITY OF ARKANSAS AT PINE BLUFF, PINE BLUFF, AR, 71601
‡ CCDC ARMY RESEARCH LABORATORY, FCDD-RLS-CB, ADELPHI, MD 20783

Abstract

Protein Catalyzed Capture (PCC) agents are synthetic, peptide-based ligands using synthetic epitope target proteins, or SynEp, screened against one-bead-one-compound (OBOC) libraries. The screening process promotes the formation of covalent bonds between SynEp and the OBOC library via in situ azide-alkyne click chemistry for a selected few, resulting in high affinity and selectivity towards target protein. PCCs have been used in studies as alternatives to antibodies in protein detection because of their proven nature of high sensitivity, selectivity, and affinity. In this study, PCC agents are used as a biosensing device to capture the Coronavirus Disease 2019 (COVID-19) virus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). COVID-19 is a global pandemic that has few tests available and diagnoses are not instant. A PCC-based biosensing device will permit for real time detection of SARS-CoV-2. In this research, PCC is attached to the surface of the bioreceptor by covalent immobilization. An azide group attached to a Silicon Nanowire (SiNW) surface through silane chemical modification allows for a stable bond between SiNW and the PCCs. PCC agents are most preferred in this case because of their strong stability in various thermal and chemical environments as well as being cheaper and more time efficient to produce.

Biosensors

- Biosensors have been studied and used for biological applications. Popular biosensors are pregnancy tests and blood glucose monitors.
- The basic structure of a biosensor is composed of two parts, a bioreceptor and a transducer.
- A bioreceptor is some sort of biological element that is used to attract the desired molecule. In this case, the PCC agents.
- A transducer must transfer the signal from the bioreceptor to an electronic one so that the device may show physical results



The basic design of a biosensor.

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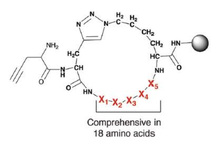
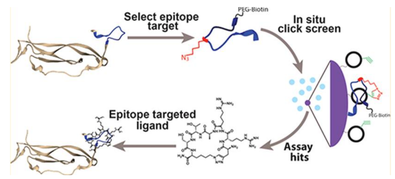
Protein Catalyzed Capture Agents as Bioreceptor

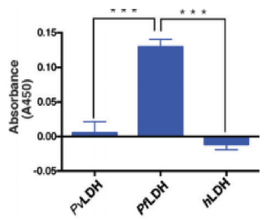
- Protein Catalyzed Capture (PCC) agents are linear or cyclic peptides containing a 5-mer variable region and a click handle attached to resin beads. The one-bead-one-compound libraries (OBOC) were synthesized using the split and mix method resulting in 2-million different sequences on the beads.
- Then a small unique section on the target protein, normally a 10-20 amino acid length peptide fragment, is chosen and synthesized with a click handle on it. This epitope is called SynEp.
- Incubating the OBOC library with the SynEp results in the formation of covalent bonds through in situ click chemistry to only a select few library members to the target.
- PCC agents developed through an extensive screening process allow them to be very selective towards that specific protein target.

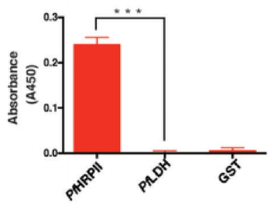




A scheme of a cyclic peptide library and the process of creating a Protein Catalyzed Capture Agent. (Referenced from Agnew, 2019)

High selectivity of PCC agents

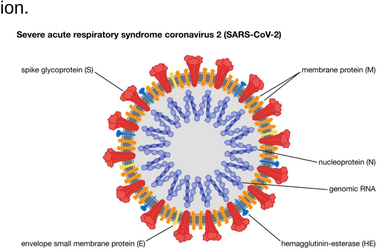


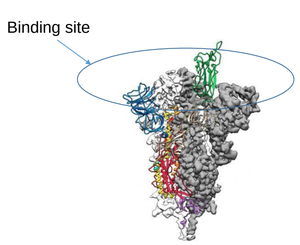


The epitope targeting shows great selectivity towards plasmodium falciparum lactate dehydrogenase (PfLDH) not towards other LDH variants on the left. On the right a different epitope targeted for plasmodium falciparum histidine-rich protein-2 (PfHRPII) but not to other molecules in the plasmodium falciparum family. (Referenced from Das, 2015)

Protein Catalyzed Capture (PCC) Agents to Detect SARS-CoV-2

- SARS-CoV-2 is the virus for the COVID-19 disease.
- Coronavirus infectious disease 2019, pictured below, has spike glycoproteins (S) on the surface. SARS-CoV-2 enters into human cells through S protein binding to ACE2 on host cells.
- Those binding sites on S protein were chosen as the target epitopes to develop PCC agents.
- PCC based SARS-CoV-2 detector would give quicker results (in minutes).
- PCC based SARS-CoV-2 detector provides high affinity, selectivity and stability and is suitable for harsh/battlefield condition.

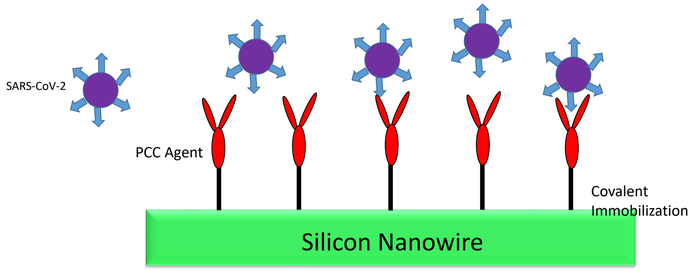




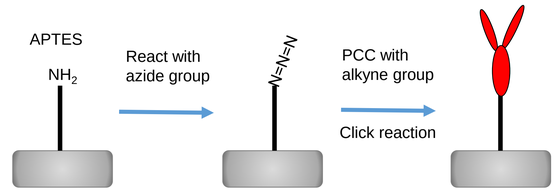
The structure of SARS-CoV-2 and the spike glycoprotein are pictured. (Referenced from Wrapp, 2020)

Structure of PCC-based Bioreceptor to Detect SARS-CoV-2

- The surface used is silicon nanowire due to the successful use of detecting other biomarkers in the body.
- Nanowire has been able to detect at very low concentrations allowing positive results sooner.
- The PCC agent is attached to the surface and attracts the SARS-CoV-2



Mechanism of attaching PCC to Silicon nanowire surface



Covalent immobilization using APTES attached to a surface, converting amine group to azide group for click reaction with alkyne group on PCCs.

- PCC is covalently immobilized onto the surface of the silicon wire.
- Covalent immobilization allows for a stable bond between the surface and the PCC Agent.
- The alkyne group on the PCC will be chemically bonded to an azide group to be attached to the surface.
- The azide is attached to the silicon Nanowire through modification of the silicon dioxide layer with 3-aminopropyltriethoxysilane (APTES)
- APTES reacts with the hydroxyl group on the silicon surface

Advantages of PCC-based receptor over traditional antibody-based receptors

- PCC agents are 1/40th the size of monoclonal antibodies.
- Antibodies are costly and take months to create.
- PCC agents can be mass produced using all robots, making it robust. Development time: approximately 2 weeks.
- PCC agents have limited batch-to-batch performance variability.
- PCC agents are stable in high and low pH ranges as well as hot and cold temperatures.
- PCC agents are easily functionalized for surface attachment, tagging.

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